Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas chromatography–mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines—A comparative study

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ABSTRACT

In this study the feasibility of different extraction procedures was evaluated in order to test their potential for the extraction of the volatile (VOCs) and semi-volatile constituents (SVOCs) from wines. In this sense, and before they could be analysed by gas chromatography–quadrupole first stage masss spectrometry (GC–qMS), three different high-throughput miniaturized (ad)sorptive extraction techniques, based on solid phase extraction (SPE), microextraction by packed sorbents (MEPS) and solid phase microextraction (SPME), were studied for the first time together, for the extraction step. To achieve the most complete volatile and semi-volatile signature, distinct SPE (LiChrolut EN, Poropak Q, Styrene-Divinylbenzene and Amberlite XAD-2) and MEPS (C_2, C_8, C_{18}, Silica and M1 (mixed C_8-SCX)) sorbent materials, and different SPME fibre coatings (PA, PDMS, PEG, DVB/CAR/PDMS, PDMS/DVB, and CAR/PDMS), were tested and compared. All the extraction techniques were followed by GC–qMS analysis, which allowed the identification of up to 103 VOCs and SVOCs, distributed by distinct chemical families: higher alcohols, esters, fatty acids, carbonyl compounds and furan compounds. Mass spectra, standard compounds and *retention index* were used for identification purposes.

SPE technique, using LiChrolut EN as sorbent (SPE$_{LiChrolut EN}$), was the most efficient method allowing for the identification of 78 VOCs and SVOCs, 63 and 19 more than MEPS and SPME techniques, respectively. In MEPS technique the best results in terms of number of extractable/identified compounds and total peak areas of volatile and semi-volatile fraction, were obtained by using C_8 resin whereas DVB/CAR/PDMS was revealed the most efficient SPME coating to extract VOCs and SVOCs from Bual wine. Diethyl malate ($18.8 \pm 3.2\%$) was the main component found in wine SPE$_{LiChrolut EN}$ extracts followed by ethyl succinate ($13.5 \pm 5.3\%$), 3-methyl-1-butanol ($13.2 \pm 1.7\%$), and 2-phenylethanol ($11.2 \pm 9.9\%$), while in SPME$_{DVB/CAR/PDMS}$ technique 3-methyl-1-butanol ($43.3 \pm 0.6\%$) followed by diethyl succinate ($18.9 \pm 1.6\%$), and 2-furfural ($10.4 \pm 0.4\%$), are the major compounds. The major VOCs and SVOCs isolated by MEPS$_{C8}$ were 3-methyl-1-butanol ($26.8 \pm 0.6\%$, from wine total volatile fraction), diethyl succinate ($24.9 \pm 0.8\%$), and diethyl malate ($16.3 \pm 0.9\%$). Regardless of the extraction technique, the highest extraction efficiency corresponds to esters and higher alcohols and the lowest to fatty acids.

Despite some drawbacks associated with the SPE procedure such as the use of organic solvents, the time-consuming and tedious sampling procedure, it was observed that SPE$_{LiChrolut EN}$, revealed to be the most effective technique allowing the extraction of a higher number of compounds (78) rather than the other extraction techniques studied.

1. Introduction

The volatile fraction of wine plays a prominent role in its organoleptic characteristics. It determines their aroma, which is the major contributor to overall flavor perception and one of the most important parameters influencing the wine quality and consumer acceptance [1,2]. Their chemical composition contains numerous small molecules (over 1000 volatile compounds) belonging to distinct chemical families, including monoterpenoids, C_{13}-norisoprenoids, sesquiterpenoids, higher alcohols, ethyl esters, fatty acids, acetates, isoamyl esters, carbonyls, sulphurs, furan compounds, and volatile phenols. Moreover, these compounds have different physicochemical properties regarding concentration (ranging from several $mg\,L^{-1}$ (e.g., ethyl acetate) to less than a

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Table 1
Basics of the considered extraction techniques.

Sorbent materials	SPE			
	Type	Interactions	Coating stability	Retention mechanism
LiChrolut EN (40–120 μm)	Porous polymer	Non polar	Hypercrosslinked	Sorption and partition
Porapak Q (50–80 mesh)	Porous polymer	Non polar	Partially crosslinked	Sorption and partition
Styrene-DVB (18–100 mesh)	Macroporous polymer	Polar	Partially crosslinked	Sorption and partition
Amberlite XAD-2 (20–60 mesh)	Porous polymer	Polar	Partially crosslinked	Sorption and partition
Sorbent materials	MEPS			
	Type	Interactions	Coating stability	Retention mechanism
Octadecyl (C$_{18}$)[a,b]	Polymer	Non polar	Partially crosslinked	Sorption and partition
Octyl (C$_8$)[a,c]	Polymer	Non polar	Partially crosslinked	Sorption and partition
Ethyl (C$_2$)[a,d]	Polymer	Non polar	Partially crosslinked	Sorption and partition
Mixed (C$_8$-SCX) M$_1$ [a,e,f]	Polymer	Bipolar	Partially crosslinked	Sorption and partition
Silica (SIL)[a,f]	Porous particle	Polar	Partially crosslinked	Sorption and partition
Coating[g]	SPME			
	Type	Interactions	Coating stability	Retention mechanism
PDMS (100 μm)	Homogeneous polymer	Nonpolar	Nonbonded	Absorption
PDMS/DVB (65 μm)	Porous particle/polymer	Bipolar	Partially crosslinked	*Adsorption*
PA (85 μm)	Homogeneous polymer	Polar	Bonded crosslinked	Absorption
PEG (60 μm)	Homogeneous polymer	Polar	Highly crosslinked	Absorption
CAR/PDMS (75 μm)	Porous particle/polymer	Bipolar	Partially crosslinked	*Adsorption*
DVB/CAR/PDMS (50/30 μm)	Porous particle/polymer	Polar	Highly crosslinked	Adsorption

[a] The base material is silica with mean particle and pore size of 50 μm and 60 Å, respectively.

[b] Low carbon load C$_{18}$; general purpose phase.

[c] Less hydrophobic than C$_{18}$; less retention of highly hydrophobic compounds; used when C$_{18}$ is to retentive.

[d] Short chain functional group is less hydrophobic than C$_8$; less retention of hydrophobic compounds; used when C$_8$ is to retentive.

[e] Mixed-mode sorbents constituted by C$_8$ and SCX copolymer.

[f] Highly polar surface; most common polar phase.

[g] PDMS, polydimethylsiloxane; PDMS/DVB, polydimethylsiloxane/divinylbenzene; PA, polyacrylate; PEG, polyethylene glycol; CAR/PDMS, CARboxen™/Polydimethylsiloxane; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane.

few ng L^{-1} (e.g., 3-isobutyl-2-methoxypyrazine, IBMP)), polarity, volatility and odour impact [3,4]. They are largely derived from four different sources: (i) the grape berry; (ii) processing of the grapes (namely crushing, pressing, etc.) by chemical, enzymatic-chemical and thermal reaction in grape must; (iii) the yeast strain used for fermentation; (iv) from containers used for wine making (wood, commonly oak) and chemical reactions during maturation wine storage [1].

Owing to the complex nature of the wine matrix, there is a consensus on that efficient sample preparation, trace-level detection and identification are important aspects of analytical methods to determine VOCs and SVOCs in wines. Many of the reported methods relies on extraction with organic solvents, including conventional techniques such as liquid–liquid extraction (LLE) [5], static or dynamic headspace extraction (SHS, DHS) [6], supercritical fluid extraction (SFE) [7], soxhlet extraction [8], and ultrasound extraction (USE) [9]. The broad polarity range of solvents and its general applicability made these techniques very popular [10]. However, most of these approaches present important drawbacks, typically are time-consuming and labour-intensive, uses of significant amounts of environmentally unfriendly solvents, and involves multi-step procedures, which can lead to analyte losses and a reduction in sensitivity. Finally, but also of importance, is the fact that many aroma compounds are chemically very unstable and can be easily oxidized or thermo degraded [4]. So, the search and development of adequate extraction techniques, that minimize the use of harmful organic solvents and/or even solvent-free procedures, and therefore more sustainable and easily implemented, has attracted the attention of many scientists. Therefore, in recent years miniaturized analytical techniques [11,12] had gained attention due to its many special features over conventional approaches [5–8]. Among many advantages, usage of little or no solvent, the low volumes of sample required, the greater sensitivity in sample preparation

than do the exhaustive extraction procedures, increasing of sensitivity of analysis and user-friendly system, should be pointed out (Table 1). So, relatively new miniaturized extraction techniques, such as microextraction by packed sorbents (MEPS), solid phase microextraction (SPME), and stir bar sorptive extraction (SBSE), have been gradually replacing conventional analytical methods.

The technique most frequently used for wine VOCs and SVOCs extraction or clean-up is SPE, which involves a liquid–solid partitioning, and the analytes are bound to active sites on the surface of solid sorbent. The possibility of using different sorbents for trapping analytes over a wide range of polarities, such as highly cross-linked copolymers, functionalized copolymers, graphitized carbons or some specific *n*-alkyl silicas, and eluents makes SPE a very selective technique (Table 1). The fact that only minor amounts of organic solvents are used compared to LLE, is why SPE has been extensively used for the analysis of volatile aroma compounds [8,13–16] and off-flavors [17,18] in wines.

More recently the conventional SPE (mL bed volumes) has been adopted for microextraction through a syringe packed with suitable solid phase material, a novel method for sample preparation and sample handling – microextraction by packed sorbent (MEPS) (μL volumes). Is a miniaturization of the conventional SPE in which the sample volume and volumes for extraction and washing solvents are reduced compared to SPE technique [19] (Table 2). A wide range of sorbents are available including C$_2$, C$_8$ and C$_{18}$ bonded phases on silica, polymeric resins (polystyrene/divinylbenzene copolymer), molecular imprinted polymers (MIPs), polar sorbents such as silica and ion-exchange sorbents, and mixed-mode sorbents. These different phases enable interactions based on adsorption, H-bonding, polar and nonpolar interactions, cation, anion exchange or size exclusion. This new technique is very promising because it is fast, simple, requires very small volume of samples (few μL) to produce comparable results to conventional SPE technique, and the cost of